Foundation for Polish Science

TEAM TECH CORE FACILITY PROGRAMME

LAUREATE ANNUAL RESEARCH PROGRESS REPORT

Project title:	**Mass Spectrometry of Biopharmaceuticals - improved methodologies for qualitative, quantitative and structural characterization of drugs, proteinaceous drug targets and diagnostic molecules.**		
Laureate:	Prof. dr hab. Michał Dadlez		
Reporting period:	from 10/2020 to 06/2021	**Period no.**	**4**
Agreement No.:	POIR.04.04.00-00-2CC8/16	from 01.11.2017 to 29.06.2021	

NOTE: Information provided within a progress report shall consider only the said project within a particular reporting period.

1. INFORMATION ON THE RESEARCH PROGRESS

1.1. Achieved deliverables and milestones (up to 1 page A4)

Please list up to 3 (three) the most important research achievements within a past reporting period:

- *Development of several new metabolomics quantitation analyses. For the Institute of Hematology and Transfusiology (IHIT) the tests for three drugs Trametinib, Gilteritinib and Dexamethason planned in clinical trial were worked out. For Jagiellonian University assay for simvastatin and its active form determination in plasma and muscle tissue homogenates were established. Also we have implemented and tested the general Pampa Protocol (Parallel Artificial Membrane Permeability Assay).*
- *Completion of a new method for quantification of FAS markers detection in meconium: the ethyl esters, ethyl glucuronide and ethyl sulfate by optimizing mass spectrometry method and chromatographical conditions.*
- *Enrichment of an in-house hydrogen deuterium exchange (HDX-MS) data program HaDeX which is available as a web server with a variety of new features. The quality of the code was improved, new types of plots (chiclet plot, butterfly plot, volcano plot) added and the documentation re-written. A novel test for simulation method for HDX-MS based on the semiparametric models with support of R-package was worked out. For prediction of cleavage sites in the HDX-MS experiments we developed a new algorithm of filtering of the k-mer data*

Please provide a brief and concise summary of the above mentioned achievements, indicating also impact of such deliverables/milestones on the overall project progress:

Deliverables mentioned complete or fulfill in part the aims of relevant tasks planned in the project at the present stage.

1.2. **Project challenges and risk assessment** (up to 1 page A4)

Please list up to 3 (three) the most important project and research challenges experienced within a given reporting period supplemented with a brief and concise information on the actions taken:

Pandemic still blocked normal communication channels and broken supply chains to some extent, substitute measures were being taken, using internet communication, and alternative sources of consummables etc. Due to that difficulties met do not exceed the routine level of scientific conduct and are of technical or merit-based nature.

Please provide a brief and concise information on the obstacles in the project implementation - research and managerial – that are likely to occur within a forthcoming reporting period and your strategy to respond and mitigate:

No obstacles

1.3. **Socio-economic impact of the project and its tech-transfer potential** (up to 1 page A4)

Please provide information on how the results achieved during the project implementation influence the key scientific, technological and socio-economic challenges of the modern world.

Many of the procedures worked out during the project and offered by the Laboratory directly serve the society, like drug level monitoring, carried out for Polish patients after transplants by the Laboratory since 2011. Similarly, procedures being worked out in frame of the project serve for medically-oriented studies and have the direct application potential also for direct use in medicine. They are often worked out in close collaboration with medical teams which ensures their fast transfer to application stage. This year for instance we established cooperation with the Institute of Hematology and Transfusiology. We will take part in the clinical trial on the use of an additional drug (Dexamethasone) to bypass resistance to Trametinib and Gilteritinib in the treatment of relapsed or refractory B-cell acute lymphoblastic leukemias. For dr Andżelika Borkowska – Medical University of Gdansk, we carry out profiling of N-homocysteinylation in ferritin originating from human umbilical vein. With dr hab. Ewa Grzybowska - Maria Skłodowska-Curie Institute of Oncology, we work on proteomic quantitative analysis of cell line HL60 with and without HAX1 protein knockout

1.4. **Detailed description of the research activities** (up to 6 pages A4)

This section shall provide a thorough information on the progress of the research programme in the past reporting period. In case of consortium, sections shall be sub-divided between two members of the consortium.

[…]

- *Global Proteomics – a new protocol has been worked out dedicated to comparative analysis of methionine oxidation levels. Methionine oxidation is a common artifact of mass spectrometric methods, at the same time, it can carry a significant biological function like in bone formation. Results obtained with this protocol in the study of cytoplasmic polyadenylation by TENT5A were presented in the publication. Established before protocols for proteogenomic and peptidomics approaches were applied in a common project with Dr Urszula Zielenkiewicz. Currently, all planned samples from one of the three tested systems, i.e. the sewage treatment plant have been fully analyzed. A database of protein sequences for this metamicrobiome has been established. The other two systems: hydrogen and methane bioreactors are currently under investigation.*

With prof. Joanna Kufel we are applying peptide-wise proteomic protocols to study alternative transcription/translation events in yeast model system in adaptation to stress conditions. Optimisation tests of LMW fraction extraction protocols from yeast cultures are on-going.

Due to the purchase and installation of the new LC-MS Evosep One / Exploris 480 system, multi-stage overall system tests were carried out using a complex mixture of peptides. The tests, in addition to thoroughly checking the operation of the new LC-MS system, were aimed at developing basic DDA (Data-Dependent Acquisition) measurement protocols and comparing the quality of the obtained results for the new equipment and systems previously owned by the Laboratory.

DIA analysis

As part of the activities carried out within Team Tech Core, in 2019 DIA (Data-Independent Acquisition) was introduced as a new analytical technique available at the Laboratory. Despite the optimization of the basic measurement and data analysis protocols, due to the insufficient quality of the MS systems, no DIA measurements for external entities were carried out in 2019-2020. In February 2021, the Institute purchased the latest Orbitrap spectrometer – Exploris 480, which allowed for the further development of the technique and its introduction to the range of services offered by the Laboratory. For the new, faster spectrometer, the basic DIA measurement parameters were once again optimized, such as: resolution, AGC target values, injection time, number of m/z windows, window width, window overlap. The parameters were set for the acquisition length of 44 min and 88 min, which correspond to the gradients offered by the Evosep One LC system. Using optimized protocols in May 2021, in cooperation with Maria Sklodowska-Curie National Research Institute of Oncology and the Laboratory of Gene Expression Regulation of IBB PAS, measurements were carried out to check the secretion of interleukins by CD4 + lymphocytes cultured in the presence of cancer cells. Obtained results were additionally compared with those obtained by means of classic DDA (data-dependent analysis) measurements. The results of the analysis became part of the manuscript: "PD-L1 overexpression, SWI / SNF complex Deregulation and Profound Transcriptomic Changes Characterize Cancer-Dependent Exhaustion of Persistently Activated CD4 + T Cells" Jancewicz et al. currently pending approval by "Cancers" journal.

iTRAQ analysis

For the new LC-MS system, a data acquisition method was developed for samples labelled with iTRAQ regents. Thanks to the much higher scanning speed of the Exploris 480, it was possible to use the Top 40 methods, compared to the previously used for the QExactive spectrometer Top 12. To reduce ion isolation interference, the PrecursorFit filter was introduced into the method, which reduces the number of fragmented peptides and thus reduces the ratio flattening effect, which is often observed in isobarically labelled samples. The developed protocol was used for the analysis of plasma samples

of patients with epilepsy under the Strategmed grant implemented in cooperation with the Medical University of Warsaw and for the analysis of HL60 cells with the HAX1 knockout in cooperation with Maria Skłodowska-Curie Institute of Oncology.
TMT and TMTPro analysis
The measurement method was optimized for samples labelled with TMT 10-plex tags, which were previously measured in the Laboratory on QExactive spectrometers. Moreover, a method of preparation and data acquisition of samples labelled with new TMTPro (Thermo Scientific) tags was introduced, enabling the simultaneous quantitative analysis of up to 16 samples. Due to the high mass spectrometer requirements, in the previous years it was not possible to analyse such samples. Multiple test measurements were carried out for 16-plex samples, which finally allowed to obtain a method with sufficient resolution to distinguish individual reporter ions while maintaining a high number of identified peptides and proteins. Optimized acquisition methods for TMT 10-plex and 16-plex have already been used to conduct several experiments, including a collaboration with prof. Nina Smolińska from University of Warmia and Mazury in Olsztyn and with dr. Joanna Nynca from Institute of Animal Reproduction and Food Research, Polish Academy of Sciences.

- *Metabolomics. Development of several new metabolomics quantitation analyses, mainly of drugs or their metabolites. These procedures are customised according to collaborator's needs. For the Institute of Hematology and Transfusiology (IHIT) the tests for three drugs Trametinib, Gilteritinib and Dexamethason planned in clinical trial were worked out. The trial will check the use of dexamethasone to avoid resistance to trametinib and gilteritinib to treat recurrent or resistant acute lymphoblastic leukemia from LiMFO B. The three drugs presented different levels of difficulty, but finally we developed novel protocol based on the Waters Ostro phospholipids removal plates for trametinib and an increased sensitivity protocol for dexamethason. Significant efforts have accompanied the implementation of the Pampa Protocol (Parallel Artificial Membrane Permeability Assay) which is now included into lab portfolio. For FAS markers detection in meconium, task initiated during the third period and was continued and completed during this one. This task will be implemented in cooperation with dr Ewa Głuszczak- Idziakowska and prof. dr hab. Bożenia Kociszewska-Najman (The Medical University of Warsaw).*

- *Structural MS. The team involved in HDX-MS data cooperated in improvements of HaDeX software being developed in frame of the project. New features have been implemented originating from feedback of programme users and literature search, especially new forms of data visualisation. Special focus was for comparing the power of the statistical tests verifying differences in deuteration levels a key task in typical HDX-MS experiments which aim at identifying regions of protein involved in a protein complex under study. Several tests, from different software packages were compared on the uniform datasets. Error analysis and hypothesis testing are an Achilles heel of the existing HDX data analysis programs. The developed power analysis has shown its better performance in comparison to the state-of-art methods. This was supported by a novel approach for HDX data modelling employing semiparametric models, Markov chains, R-package and k-mer analysis.*

2. PROJECT PROMOTION AND DISSEMINATION OF THE RESULTS

COVID pandemic state does not allow for most effective promotion by personal contacts and participation in scientific events. These activities were largely stalled starting march 2020. Instead, other means of communication, mails, web page, personal contacts, social media served to disseminate the knowledge on new possibilities offered thanks to CORE financing and gained new clients from BioPharma companies (Neutrino Geology, Spinprot, CAPTOR, FiLeClo, Selvita S.A., PureBiologics S.A., and scientific institutions (prof Przemysław Juszczynski (IHiT), prof. Marszałek from Gdańsk Univ., prof. Pokrzywa IIMCB, Warsaw, prof. Chacińska, CENT, Warsaw, prof. Rodziewicz-Motowidło, U.Gd., Prof. F. Sobott, Leeds Univ.)

3. PARTNERSHIP IN THE PROJECT

3.1. Description of collaboration with foreign research partner(s)

This section shall provide a brief and concise information on the nature of collaboration with foreign research partner.

[…]

Numerous long-lasting international collaborations with foreign partners described in previous reports are on–going, for instance in the areas of structural studies of proteins by MS, leading to a series of new publications with prof. prof. D. Glover, Caltech, Cambridge Univ., B. Negrutskii, MMCB, Kiev, Z. Domiński, North Carolina Univ., H. Herrmann, DKFZ Heidelberg, and sparked new collaborative efforts like with prof. F. Sobott, Leeds Univ., dr. M. Kulma, Slovenian Acad. Sci.. New proteomic approaches are used in Zika virus capsid-protein interactome co-immunoprecipitation experiments are performed with two teams lead by prof. Michał Hetman and prof. Dong Chung form Kentucky University.

3.2. Description of collaboration with local research partner(s)

This section shall provide a brief and concise information on the nature of collaboration with local research partner.

[…]

Numerous collaborations with local partners, described in previous reports are on–going. New collaborations include:

Dr. Ulrike Topf (IBB-PAS): Study of ribosomal structures. Targeted proteomic analysis of ribosomal protein components. Interactomics in *S. cerevisiae*.

Dr. Grażyna Dobrowolska (IBB-PAS): Targeted proteomic analysis of phosphorylations to study stress responses in *Arabidopsis thaliana.*

With prof. Joanna Kufel we are applying peptide-wise proteomic protocols to study alternative transcription/translation events in yeast model system in adaptation to stress conditions. Optimisation tests of LMW fraction extraction protocols from yeast cultures are on-going.

Prof. Wojciech Łaba – Wrocław University of Environmental and Life Sciences. Plant peptides identification in brewer's spent grain.

Michał Marcinkowski – Neutrino Geology. Tracing the biological source of catalase sample.

Prof. dr hab. Nina Smolińska - University of Warmia and Mazury in Olsztyn, Proteomic quantitative profiling of *Sus scrofa domestica* reproductive tissues.

Dr Joanna Nynca - Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Quantitative analysis of di-and triploid ovaries of rainbow trout.

Dr Andżelika Borkowska – Medical University of Gdansk, Profiling of N-homocysteinylation in ferritin originating from human umbilical vein.

Martyna Szczepara - University of Wrocław, Interactomics of differentially transfected U2OS cells.

Wojciech Woźny – Spinprot, Protein contamination analysis of hGH sample.

Dr hab. Ewa Grzybowska - Maria Skłodowska-Curie Institute of Oncology, Proteomic quantitative analysis of cell line HL60 with and without HAX1 protein knockout.

Dr Agata Cieśla - Laboratory of Biotechnology Faculty of Biology, AMU, Identification of SUMOylation sites in ACS7 protein from *Arabidopsis thaliana*.

Krystian Hyszko – Warsaw University, Identification of *Arabidopsis thaliana* proteins co-purifying with DXO1.

Dr Maciej Kochanowski - National Veterinary Research Institute in Pulawy, Quantitative analysis of *Anisakis simplex* treated with essential oils.

prof. Dr hab. Katarzyna Dorota Raczyńska - Adam Mickiewicz University in Poznań, Human protein identification interacting with specific RNA.

This year we also established cooperation with the Institute of Hematology and Transfusiology. We will take part in the clinical trial on the use of an additional drug (Dexamethasone) to bypass resistance to Trametinib and Gilteritinib in the treatment of relapsed or refractory B-cell acute lymphoblastic leukemias. Specifically, for prof Przemysław Juszczynski (IHiT), we collaborate on new, non-canonical functions of PIM kinases in lymphomas, by listing proteins for which phosphorylation becomes limited by inhibitors in cancer cells.

4. IMPACT OF THE PROGRAMME ON THE PI's CAREER

Please provide information and necessary feedback on how is this programme affecting your scientific and professional development.

Project enables transfer of research into new analytical procedures made available for a wider scientific community, but also provides new potential diagnostic tools of importance to medicine. Therefore it enables to disseminate the developments in a science oriented environment into the society. It ensures a broader perspective for the activity of the Mass

Spectrometry Lab, I have a privilege to direct. Project increased visibility and recognition of the Lab on the European level and also enabled successful applications for new infrastructure purchases for the Lab (Exploris 480, UHMR).

5. DEVELOPMENT OF RESEARCH TEAM MEMBERS

5.1. Information on the supervision of PhD students, including additional mentors

Since the aim of the project is to increase the human potential of the R&D sector, provide necessary information on the scientific and professional supervision of PhD students participating in the projects. It is crucial to provide information on mandatory supervision of PhD students by external mentors.

All students are supervised on a daily basis by the PI and postdocs due to pandemic state by remote conferences and mail correspondence. Lab meetings had to be temporarily discontinued. External mentors for PhD students are in contact with PhD students: Prof. J. Pierzynowski and prof. B. Negrutskii, they actively participate in common projects in which both PhD students are involved. Dr Michał Burdukiewicz (Warsaw Technical Univ.) is mentoring PhD student working in structural proteomics field in aspects of statistics and programing.

5.2. Supervision of research staff

Since the aim of the project is to increase the human potential of the R&D sector, provide necessary information on the scientific and professional supervision of research team members.

All students and lab members are supervised on a daily basis by the PI and postdocs in mail and remote conferences and consultations. Lab meetings, held on a weekly basis, had to be discontinued due to pandemia, they will be reinstated as soon as the situation permits. Meeting are held by communicator platforms and all discussion held by mails.

6. IMPLEMENTATION OF THE PROJECT ACCORDING TO THE PLANNED SCHEDULE

YES X NO ☐

7. CORE FACILITY DEVELOPMENT

Accordingly to TEAM-TECH Programme Core Facility Competition Documentation during the eligibility period of the project, but no later than the day preceding the commencement of the service, the regulations specifying the rules of access to the services provided by the core facility will be made available to all potential recipients; the above mentioned regulations are

subjected to consultation and approval by the Organization. Please provide the following information:

a) **The regulations specifying the rules of access to the services provided by the core facility have been prepared and approved by the Organization and are available to all potential recipients:**

YES ☐ X

If marked "YES" above, please write the website address where the regulations are available:

http://mslab-ibb.pl/en/services

b) **A price list for the services provided by the core facility has been published and is available to all potential recipients:**

YES ☐X

If marked "YES" above, please write the website address where the regulations are available:

http://mslab-ibb.pl/en/services/service-fees

c) **The number of services provided for external recipients (enterprises and other research organizations)**

More than 10000. MS Lab provides a variety of services, some require few minutes of work, while the others last for months. Therefore, any simple numbers may be misleading. For instance the number of quantitative small molecule (metabolomics) analyses in 2020 was 4878, while the number of proteomic identification or quantitation commissioned analyses was 2348. Mass measurement runs and structural proteomic analyses increase this number substantially.

The number of services provided internally (for other research groups or units at your Organization)

More than 1000. As above, a variety of analyses carried out makes this estimation not informative.

8. ADDITIONAL INFORMATION

Please provide – if necessary – all other important information relevant to the project implementation.

[…]

9. PI's WORKLOAD

I, the undersigned, hereby state that:

X My total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, did not exceed 276 hours in any month from the reporting period.

☐ My total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, exceed 276 hours in the below indicated months from the reporting period*:

Year/Month	Number of hours of the total commitment per month
Oct. 2020- Jun.2021	220

10. TEAM MEMBERS WORKLOAD:

X According to the team member's statements in no case the total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, did not exceed 276 hours in any month from the reporting period.

☐ According to the team member's statements in the following cases the total commitment to projects financed from EU Structural Funds, ESF and other activities financed from other sources, including the beneficiary's and other entity's funds, exceeded 276 hours in the below mentioned months from the reporting period*:

Dominik Cysewski

Year/month	Number of hours of the total commitment per month
2020/10	180
2020/11	170
2020/12	176

2021/01	176
2021/02	80 – on a medical leave since 10.02.2020
2021/03	Medical leave
2021/04	Medical leave
2021/05	Medical leave
2021/06	Medical leave

Emilia Samborowska:

Year/month	Number of hours of the total commitment per month
10/2020	88
11/2020	80
12/2020	76
1/2021	76
2/2021	80
3/2021	120
4/2021	168
5/2021	152
6/2021	168

Jakub Kała:

Year/month	Number of hours of the total commitment per month
2020/10	90
2020/11	90

2020/12	90
2021/01	90
2021/02	90
2021/03	90
2021/04	90
2021/05	90
2021/06	90

Katarzyna Dabrowska:

Year/month	Number of hours of the total commitment per month
2020/10	160
2020/11	170
2020/12	180
2021/01	175
2021/02	165
2021/03	170

Krystyna Grzesiak:

Year/month	Number of hours of the total commitment per month
2020/10	90

2020/11	90
2020/12	90
2021/01	90
2021/02	90
2021/03	90
2021/04	90

2021/05	90
2021/06	90

Michał Burdukiewicz:

Year/month	Number of hours of the total commitment per month
2020/10	152
2020/11	152
2020/12	152
2021/01	152
2021/02	152
2021/03	152
2021/04	152
2021/05	152
2022/06	152

Radosław Jaźwiec:

Year/month	Number of hours of the total commitment per month

10.2020	130
11.2920	130
12.2020	130
01.2021	130
02.2021	130
03.2021	130
04.2021	130
05.2021	130
06.2021	130

Weronika Puchała:

Year/month	Number of hours of the total commitment per month
10/2020	180
11/2020	160
12/2020	160
01/2021	160
02/2021	160
03/2021	180
04/2021	160
05/2021	160
06/2021	160

* In case of exceeding 276 hours monthly of the total commitment to any projects of an employee/stipendee, his/her remuneration/stipend from the Project funds will constitute an ineligible expenditure for each month in which 276 hours have been exceeded.

11. Appendixes to the merit-based annual progress report in the electronic version:

- Project realization indicators (on-line data base: *Progress reports* tab/ *progress report*/*Indicators*)
- Scientific Achievements of the Laureate and Team Members (on-line data base: *Progress reports* tab/ Publications/Patents/Academic degrees and titles/Awards and grants),
- List of conferences, scientific exchanges and business meetings (doc. file uploaded to on-line data base: *Progress reports* tab/*Progress report/Attachments*)
- Progress reports of team members – if applicable - (doc. file uploaded to on-line data base: *Progress reports* tab/*Progress report/Attachments*)

Note: The Laurate's Annual Research Progress Report should be uploaded to the Foundation's database (in the *Progress reports tab/Progress report/Attachments tab*) in two formats: as a '.doc' file and its signed, scanned version as a '.pdf' file.

I, the undersigned, hereby confirm that the information contained in the merit, periodic report (both electronic and paper version) are true. I am aware of the legal consequences of giving untrue information in a legally significant situation, as stated in article 271 of the Penal Code.

Date:……

Laureate Signature……